# EPOXYPEPTIDE ANTIBIOTIC TETAINE MIMICS PEPTIDES IN TRANSPORT TO BACTERIA

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(Received for publication July 13, 1981)

Tetaine induced the lysis of *Escherichia coli* cells. Several di- and tripeptides were found to protect this cells against tetaine action. Certain peptides are able to diminish the inhibition by tetaine of diaminopimelic acid incorporation into peptidoglycan and the extent of this corresponds to the protection of the cells against the tetaine-induced lysis. The data indicate that tetaine enters *E. coli* cells predominantly by dipeptide permease and in part by one or more oligopeptide permease system. A number of di- and tripeptides diminished the inhibitory effect of tetaine on the incorporation of lysine into peptidoglycan of *Staphylococcus aureus* Oxford. In contrast to *E. coli*, tetaine seems to be transported into *S. aureus* by a single transport system.

Tetaine, L-alanyl-L-β-(2,3-epoxycyclohexanono-4)alanine, is a dipeptide antibiotic containing an epoxyaminoacid moiety. Tetaine is synonymous with bacilysin[1,2] and bacillin[3]. The antibiotic exhibits broad antimicrobial spectrum including Gram-positive and Gram-negative bacteria[4,5] and *Candida albicans*[6].

We have established earlier that tetaine inhibits the synthesis of bacterial cell-wall peptidoglycan in *E. coli*[7] and *S. aureus*[8] at early stage and that tetaine is transported into bacterial cells by a peptide carrier system[8]. In *C. albicans* tetaine inhibits mannoprotein and chitin synthesis and is transported in the same manner as in bacteria, that is, by peptide permease[6].

KENIG and ABRAHAM[9] later showed that bacilysin is a powerful inhibitor of the enzyme L-glutamine-D-fructoso-6-phosphate amidotransferase, glucosamine synthetase, EC 2.6.1.16. These authors have also demonstrated that the active inhibitor is not bacilysin itself but its C-terminal epoxyamino acid named anticapsin. Anticapsin is generated inside the cells due to hydrolysis of bacilysin by peptidases[9]. It is suggested that the activity of bacilysin depends on its transport into the susceptible organisms[9]. The free epoxyamino acid is not transported into bacterial cells and therefore is not active on intact cells[5]. Inhibition by tetaine (its C-terminal epoxyamino acid—anticapsin) of glucosamine synthesis resulted in bacteria in the inhibition of peptidoglycan formation accompanied by a bacteriolytic effect[6].

These phenomena have been employed in our investigation on the transport of tetaine. Our aim was to provide additional evidence for the use by tetaine of peptide permease system.

## Materials and Methods

Tetaine was isolated and purified from filtrates of *Bacillus pumilus* strain "theta" in our laboratory[4].

Peptides were obtained from Sigma, St. Louis, and from Nutritional Biochemicals Co., Cleveland. All procedures and experimental conditions are given in legends to Figs. and Tables.

## Results and Discussion

As shown in Fig. 1A, in presence of tetaine the growth profiles of *E. coli* are similar to those with

Fig. 1. Bacteriolytic effect of the antibiotic tetaine on growing cells of *Escherichia coli* K-12 strain 3000 Hfr and its prevention by some peptides.

Cells growing exponentially in CGPY medium[14] at 37°C were washed twice with prewarmed saline and resuspended in 0.1% Casamino Acids (Difco)-Minimal Broth Davis (Difco) to an optical density 0.150 at 660 nm. After 15 minutes preincubation with shaking, appropriate concentrations of tetaine and of tetaine with peptides in small volumes were added. Growth with shaking at 37°C was followed by measurement of the optical density of the culture at 660 nm in Zeiss spectrocolorimeter. Growth was expressed as Klett units.

A. Tetaine at 10~160 μM
B. Tetaine, 80 μM; Ala-Ala added at 1.0 to 7.5 mM
C. Tetaine, 80 μM; dipeptides at 5 mM
D. Tetaine, 80 μM; peptides at 5 mM.

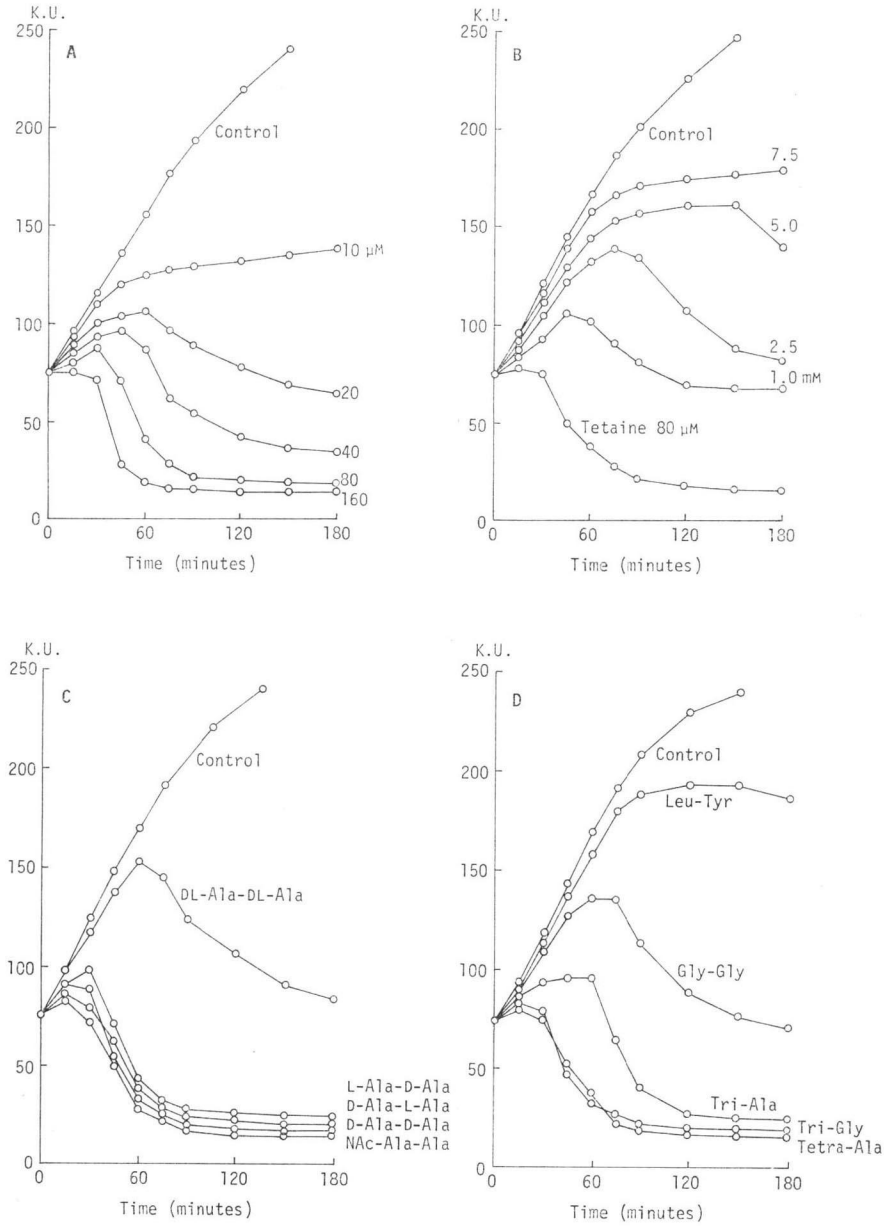

Table 1. Effect of some peptides on the delay of the onset of lysis of *E. coli* K-12 induced by antibiotic tetaine.

| Tetaine at 80 μM and peptides at 5 mM | Delay of time to lysis (minute)* |
|---|---|
| Gly-Ala | 75 |
| Gly-Met | 60 |
| Gly-ILeu | 90 |
| Gly-Trp | 90 |
| Gly-Phe | 90 |
| Gly-Tyr | 90 |
| Gly-GlyOMe | 45 |
| Gly-GlyOEt | 0 |
| Ala-Gly | 105 |
| Ala-Leu | 150 |
| Ala-Tyr | 135 |
| Tyr-Ala | 165 |
| Tyr-Leu | 180 |
| Leu-Gly | 120 |
| Leu-Ala | 165 |
| Leu-Phe | 180 |
| Gly-Gly-Ala | 15 |
| Gly-Gly-Phe | 15 |
| Gly-Gly-Leu | 30 |
| Gly-Ala-Ala | 45 |
| Gly-Leu-Tyr | 45 |
| Leu-Gly-Gly | 60 |

* Time to lysis induced by 80 μM tetaine itself was 15 minutes. Experimental conditions were the same as described in the legend to Fig. 1.

common cell-wall antibiotics. After the addition of the antibiotic, the cells continued initially to grow, though at reduced rate, followed by a rapid lysis. Tetaine caused growth inhibition without lysis only at the lowest concentration (10 μM). Time required for the initiation of cell lysis depended reciprocally on the antibiotic concentration and ranged from 30 to 15 minutes at tetaine concentrations from 80 to 160 μM. Addition of Ala-Ala to a culture simultaneously with tetaine prolonged the time to lysis (Fig. 1B). This response depended on the concentration of the dipeptide. A clear effect was observed at 1 mM Ala-Ala, while at 7.5 mM Ala-Ala no lysis of *E. coli* cells was observed (Fig. 1B). As shown in Fig. 1C dipeptides in other than LL

Table 2. The influence of peptides on the inhibition by tetaine of incorporation of labelled amino acids into peptidoglycan of *E. coli* K-12 strain 3000 Hfr and *S. aureus* Oxford.

| Tetaine at 50 μM and peptides at 5 mM | | % Inhibition of incorporation | |
|---|---|---|---|
| | | *E. coli* K-12 $^3$H-DAP | *S. aureus* Oxford $^3$H-Lys |
| Tetaine | | 84 | 76 |
| and | Gly-Ala | 71 | 59 |
| | Gly-Pro | 72 | 57 |
| | Gly-Tyr | 52 | 45 |
| | Gly-Phe | 56 | 48 |
| | Ala-Ala | 37 | 39 |
| | L-Ala-D-Ala | 83 | 71 |
| | D-Ala-L-Ala | 87 | 78 |
| | D-Ala-D-Ala | 85 | 74 |
| | NAc-Ala-Ala | 85 | 76 |
| | Ala-Leu | 23 | 22 |
| | Ala-Tyr | 32 | 35 |
| | Leu-Ala | 9 | 3 |
| | Leu-Tyr | 5 | 4 |
| | Tyr-Ala | 19 | 9 |
| | Tyr-Leu | 7 | 7 |
| | Gly-Gly-Gly | 82 | 64 |
| | Gly-Gly-Ala | 71 | 42 |
| | Gly-Gly-Phe | 65 | 24 |
| | Gly-Gly-Leu | 57 | 28 |
| | Gly-Ala-Ala | 44 | 22 |
| | Leu-Gly-Gly | 40 | 12 |

Cells growing exponentially in CGPY medium[14] at 37°C were washed twice with prewarmed CWSM I medium[14] and resuspended in the same medium to an optical density of 0.5 at 660 nm. CWSM I medium for *E. coli* was supplemented with 5 μg/ml unlabelled diaminopimelic acid and 100 μg/ml chloramphenicol; for *S. aureus* 5 μg/ml unlabelled lysine, 100 μg/ml glycine and 50 μg/ml chloramphenicol were added. After 15 minutes preincubation of tetaine and peptide were added. 2 μCi/ml $^3$H-diaminopimelic acid (specific activity 332 mCi/mmole) and 2 μCi/ml L-$^3$H-lysine (specific activity 5.5 Ci/mmole) were added to the cultures of *E. coli* and *S. aureus*, respectively.

After 60 minutes of incubation with shaking at 37°C, aliquots of 0.25 ml were taken into ice-cold 3% perchloric acid. After 30 minutes samples were filtered through glass-fibre filters GF/C, filters were washed, dried and counted. Per cent of inhibition was calculated in relation to control without antibiotic. Incorporation into the control cells was 29,500 cpm/0.25 ml sample and 60,800 cpm/0.25 ml sample for *E. coli* and *S. aureus*, respectively.

configuration can not protect the cells against the tetaine action; *N*-acetylated Ala-Ala is also ineffective.

Data from Fig. 1D and Table 1 indicate that besides Ala-Ala a variety of other di- and tripeptides prevent the action of tetaine. In general, the peptides containing an *N*-terminal amino acid residue other than glycine are able to diminish the bacteriolytic effect of tetaine. Especially Leu-X peptides are highly effective. A number of tripeptides (except triglycine and trialanine) prevented the bacteriolytic effect of tetaine (Table 1), though they were less effective than dipeptides at the same concentration (Fig. 1D, Table 1).

Data from the bacteriolytic studies are in accordance with the observation that certain peptides are able to prevent the inhibition by tetaine of diaminopimelic acid incorporation into peptidoglycan of *E. coli*. The results summarized in Table 2 are comparable with the lysis-preventing effects of peptides. Di- and tripeptides prevented to various degree the inhibitory action of tetaine. The best protection was exerted by the di- and tripeptides containing Leu in the *N*-terminal position. In general, tripeptides were less effective than dipeptides.

The above data indicate that the transport of tetaine in *E. coli* K-12 obeys the structural requirements for peptide transport in bacteria[10], and tetaine enters the cells predominantly by dipeptide permease and in part by one or more oligopeptide permease system. (The evidence for a multiplicity of oligopeptide transport system in *E. coli* is well known[11].)

Thus, our findings support those by DIDDENS and coworkers[12] who found by use of *E. coli* mutants with modified transport activities that bacilysin enters into the cells *via* dipeptide permease and in part *via* oligopeptide permease systems.

As shown in Table 2 numerous di- and tripeptides diminish the inhibitory effect of tetaine on the incorporation of labelled lysine into peptidoglycan of *S. aureus* Oxford. In contrast to *E. coli* a number of di- and tripeptides compete to the same extent with the action of tetaine in *S. aureus*. This indicates that all these peptides may share a single transport system in *S. aureus*. Also PERRY and ABRAHAM have shown that bacilysin is taken up by sensitive *S. aureus* NCTC 6571 using the only peptide transport system in this organism common for di- and oligopeptides[13].

The above results enable us to draw the following conclusions:

1. Tetaine mimics dipeptides in entering bacterial cells *via* peptide carrier systems and is able to use both dipeptide and oligopeptide permeases.

2. Tetaine can be used as a probe for characterization of peptide transport systems in sensitive Gram-positive and Gram-negative bacteria. The simple tests can be based on the monitoring of tetaine induced secondary effects (peptidoglycan synthesis inhibition and bacteriolysis) and their prevention by peptides.

Acknowledgements

The authors acknowledge the financial support of these studies by the Institute of Organic Chemistry, Polish Academy of Sciences, Warsaw, Poland.

## References

1) WALKER, J. E. & E. P. ABRAHAM: The structure of bacilysin and other products of *Bacillus subtilis*. Biochem. J. 118: 563~570, 1970

2) KAMIŃSKI, K. & T. SOKOŁOWSKA: The probable identity of bacilysin and tetaine. J. Antibiotics 26: 184~185, 1973
3) ATSUMI, K.; R. ŌIWA & S. ŌMURA: Production of bacillin by *Bacillus* sp. strain No. KM-208 and its identity with tetaine (bacilysin). J. Antibiotics 28: 77~78, 1975
4) KRYŃSKI, S. & E. BECLA: The bacteriology of tetaine. Acta Microbiol. Polon. 12: 131~139, 1963
5) KENIG, M. & E. P. ABRAHAM: Antimicrobial activities and antagonists of bacilysin and anticapsin. J. Gen. Microbiol. 94: 37~45, 1976
6) CHMARA, H.; M. SMULKOWSKI & E. BOROWSKI: Growth inhibitory effect of amidotransferase inhibition in *Candida albicans* by epoxypeptides. Drugs Exptl. Clin. Res. 6: 7~14, 1980
7) CHMARA, H. & E. BOROWSKI: Antibiotic tetaine, a new inhibitor of murein precursor synthesis in *Escherichia coli* K-12. Biochem. Biophys. Res. Comun. 52: 1381~1385, 1973
8) CHMARA, H. & E. BOROWSKI: The inhibition of murein synthesis in *Staphylococcus aureus* by the antibiotic tetaine. Biochem. Biophys. Res. Commun. 55: 1147~1155, 1973
9) KENIG, M.; E. VANDAMME & E. P. ABRAHAM: The mode of action of bacilysin and biochemical properties of bacilysin-resistant mutants. J. Gen. Microbiol. 94: 46~54, 1976
10) BARAK, Z. & C. GILVARG: Peptide transport. *In* "Biomembranes", Vol. 7, pp. 167~218, *Ed.* L. A. MANSON, Plenum Press, London
11) NAIDER, F. & J. M. BECKER: Multiplicity of oligopeptide transport systems in *Escherichia coli*. J. Bacteriol. 122: 1208~1215, 1975
12) DIDDENS, H.; M. DORGERLOH & H. ZÄHNER: Metabolic products of microorganisms. 176. On the transport of small peptide antibiotics in bacteria. J. Antibiotics 32: 87~90, 1979
13) PERRY, D. & E. P. ABRAHAM: Transport and metabolism of bacilysin and other peptides by suspension of *Staphylococcus aureus*. J. Gen. Microbiol. 115: 213~221, 1979
14) LUGTENBERG, E. J. J. & P. G. deHAAN: A simple method for following the fate of alanine containing components in murein synthesis in *Escherichia coli*. Antonie van Leeuwenhoek J. Microbiol. Serol. 37: 537~552, 1971